

● PRINTER RUSH ●

(PTO ASSISTANCE)

IFW

Application : <u>09/205664</u>	Examiner : <u>Fredman</u>	GAU : <u>1634</u>
From : <u>TW</u>	Location : IDC FMF <u>FDC</u>	Date : <u>6-24-05</u>
Tracking # : <u>5888588</u>		Week Date : <u>1-12-04</u>

DOC CODE	DOC DATE	MISCELLANEOUS
<input type="checkbox"/> 1449	_____	<input type="checkbox"/> Continuing Data
<input type="checkbox"/> IDS	_____	<input type="checkbox"/> Foreign Priority
<input type="checkbox"/> CLM	_____	<input type="checkbox"/> Document Legibility
<input type="checkbox"/> IIFW	_____	<input type="checkbox"/> Fees
<input type="checkbox"/> SRFW	_____	<input type="checkbox"/> Other
<input type="checkbox"/> DRW	_____	
<input type="checkbox"/> OATH	_____	
<input type="checkbox"/> 312	_____	
<input checked="" type="checkbox"/> SPEC	<u>3-13-01</u>	

[RUSH] MESSAGE: _____

On Page 24 of the specification dated 3-12-01 there is data missing on Line 18.

Please correct

Thank You
TW

[XRUSH] RESPONSE: Done

INITIALS: BP

NOTE: This form will be included as part of the official USPTO record, with the Response document coded as XRUSH. Doc. # 47544-2001210 6/28/04 Burnett

REV 10/04

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09/805,664

Example 2**Identification of Proliferation Genes by Antisense Inhibition**

Seven clones obtained as described in Example 1 inhibited bacterial proliferation and
5 contained gene fragments in an antisense orientation.

Three of the clones contained fragments of the *lepB* gene; pJB12, pJB37 and pJB40.
pJB37 contains a 546 bp *HindIII* - *PstI* insert that expresses an RNA complementary to the
carboxy terminal half of the *lepB* mRNA. The RNA sequence is shown in Figure 1. *LepB*
encodes the leader peptidase responsible for proteolytic cleavage of the signal peptide from
10 preproteins. Leader peptidase is required for viability and a temperature sensitive *lepB* mutant
lyses at the nonpermissive temperature.

A fragment of an uncharacterized ORF was cloned twice in antisense orientation in
pJB39 and pJB53. The antisense clone pJB53 contains a 714 bp *PstI* - *HindIII* fragment of this
putative ORF, designated herein as *viaA* (viability inhibited by antisense) and expresses an RNA
15 that is complementary to the putative sense RNA. The sequence of the antisense RNA is shown
in Figure 2 and the sequence of the DNA insert is shown in Figure 3. Plasmid pJB53 has been
deposited with the American Type Culture Collection (ATCC), Rockville, Maryland as accession
number _____.

One antisense clone, pJB57, contained a fragment of the *ddlB* gene. The D-alanyl:D-
20 alanine ligase antisense clone, pJB57, contains a 1050 bp *EcoRI* - *BamHI* fragment (with an
internal *EcoRI* site) that expresses an RNA antisense to the carboxy terminal portion of *murC*
and the first 500 bases of *ddlB* including its start codon. The RNA sequence is shown in Figure
4. The product of the *ddlB* gene, the D-alanyl:D-alanine ligase, ligates 2 D-alanines together

(original page 24)

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20 alanine ligase antisense clone, pJB57, contains a 1050 bp *EcoRI* - *BamHI* fragment (with an
internal *EcoRI* site) that expresses an RNA antisense to the carboxy terminal portion of *murC*
and the first 600 bases of *ddlB* including its start codon. The RNA sequence is shown in Figure
4. The product of the *ddlB* gene, the D-alanyl:D-alanine ligase, ligates 2 D-alanines together